

Fig. 1

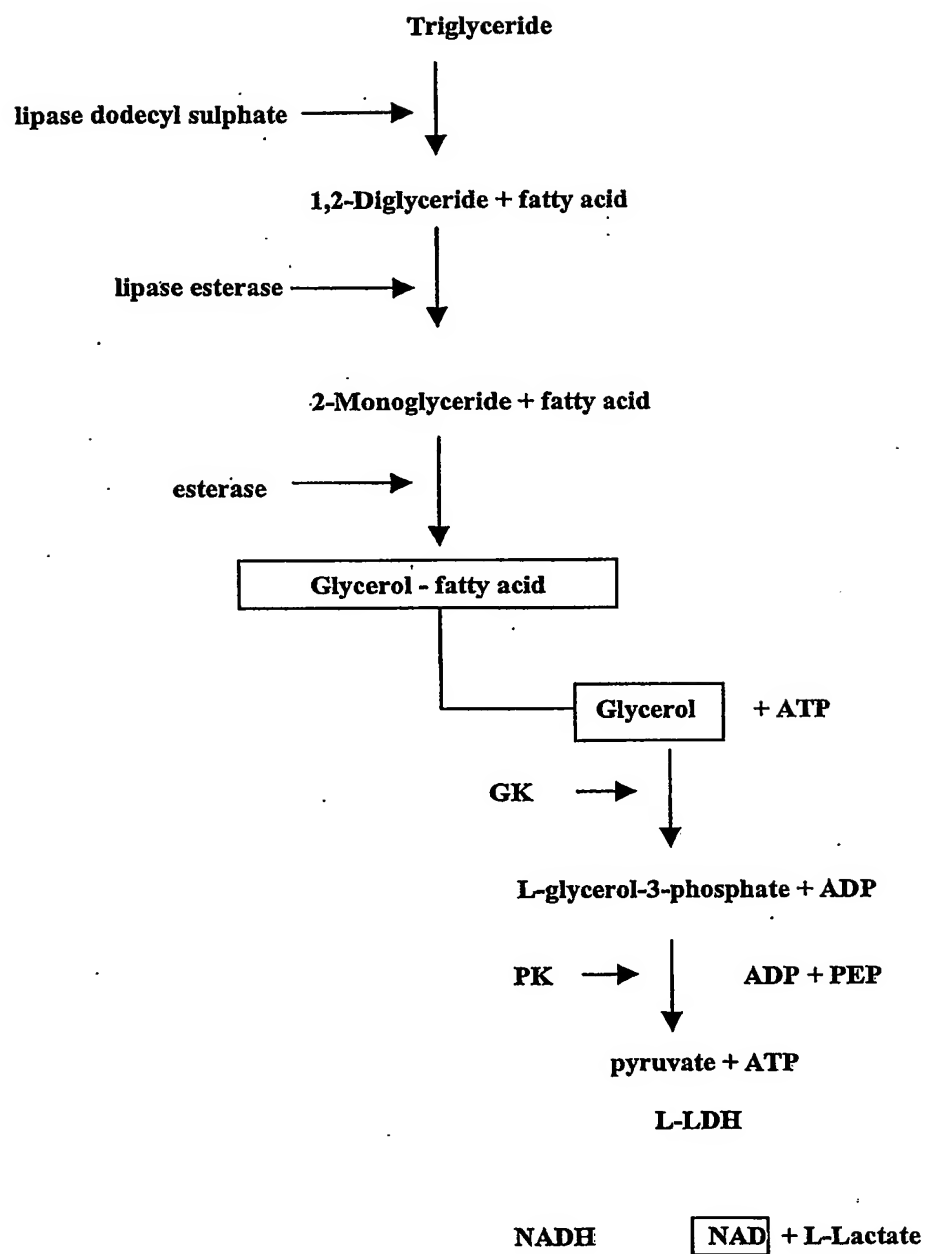


Fig. 2

Novel human membrane glycoprotein

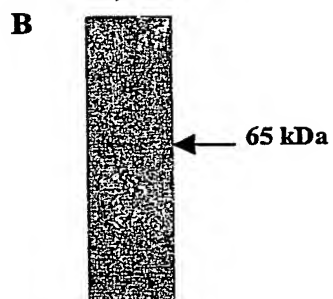
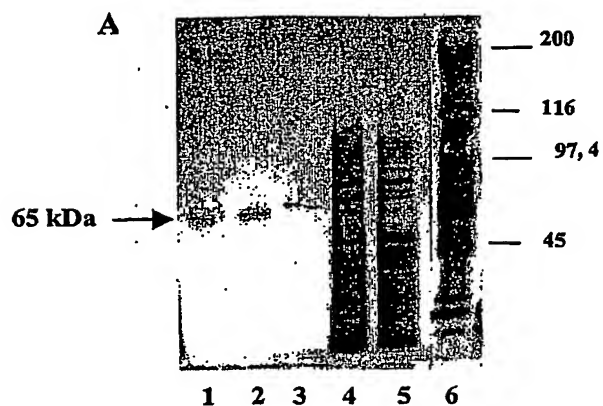


Fig. 3

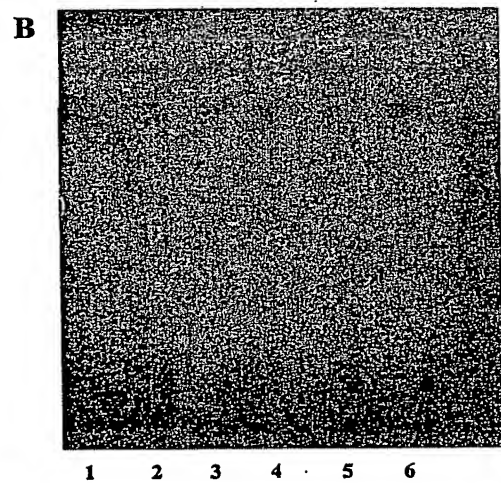
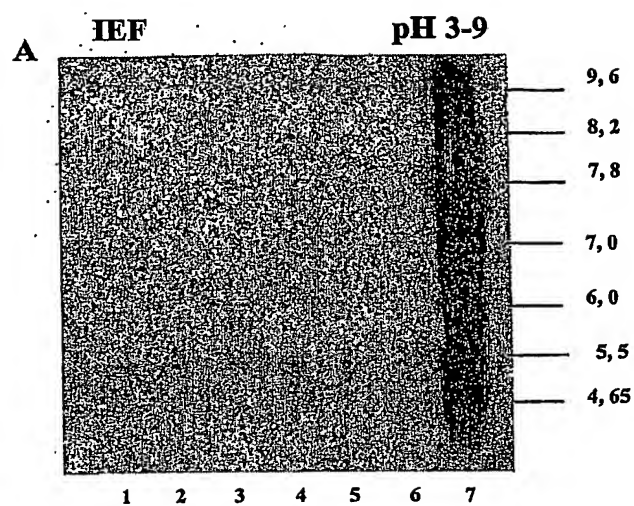


Fig. 4

Peptide A: D-L-V-P-L-E-D-K-V-T-I-L-G-M-T-A

Peptide B: K-L-A-L-S-A-D-D-P-G-F-H-N-F-S-H-Q-R-Q-T

Peptide C: D-Q-Q-T-T-S-H-S-S

Peptide D: V-L-E-I-M-L-P

Peptide E: F-Q-D-E-S-E-A-N-K

Peptide F: M-K-Y-V-N-F-K-F-Y-F

Peptide G: N-L-D-F-M-T-W-G-V-T-K-V-T-Y-I-G-Q-P-T-G-G

Peptide H: L-L-M-D-N-N-E-A-V-H

Peptide I: F-D-Q-A-W-A-D-T-A-H-T-W

Peptide J: K-L-D-D-I-Q-K-D-M-Y-S-Q-Q-D-T

Peptide K: G-V-W-I-M-K-N-Q-I-T

Fig. 5

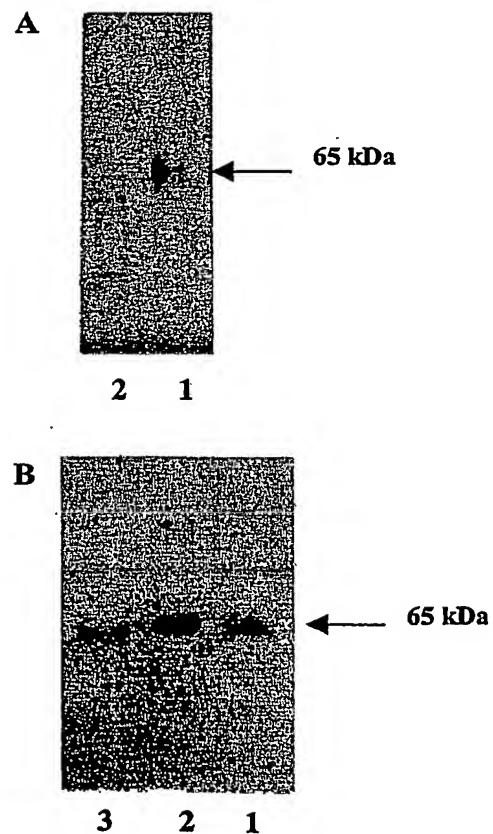


Fig. 6

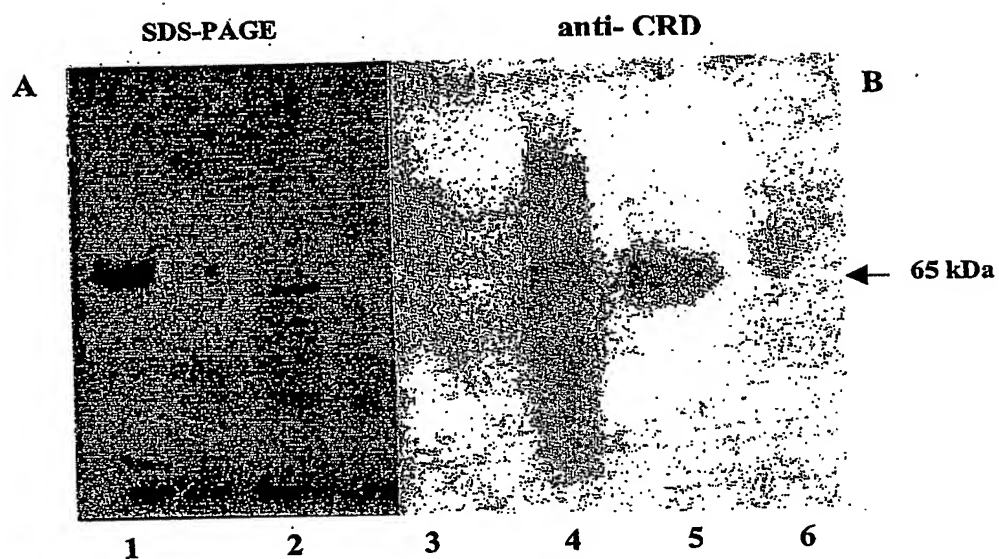


Fig. 7

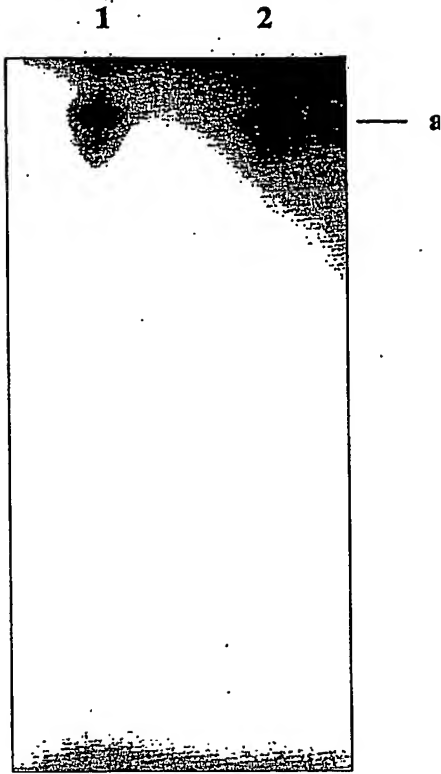
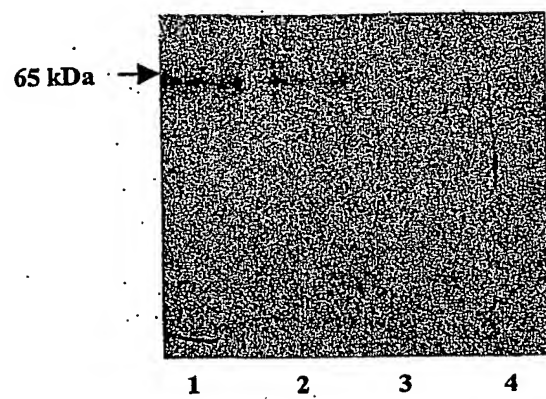
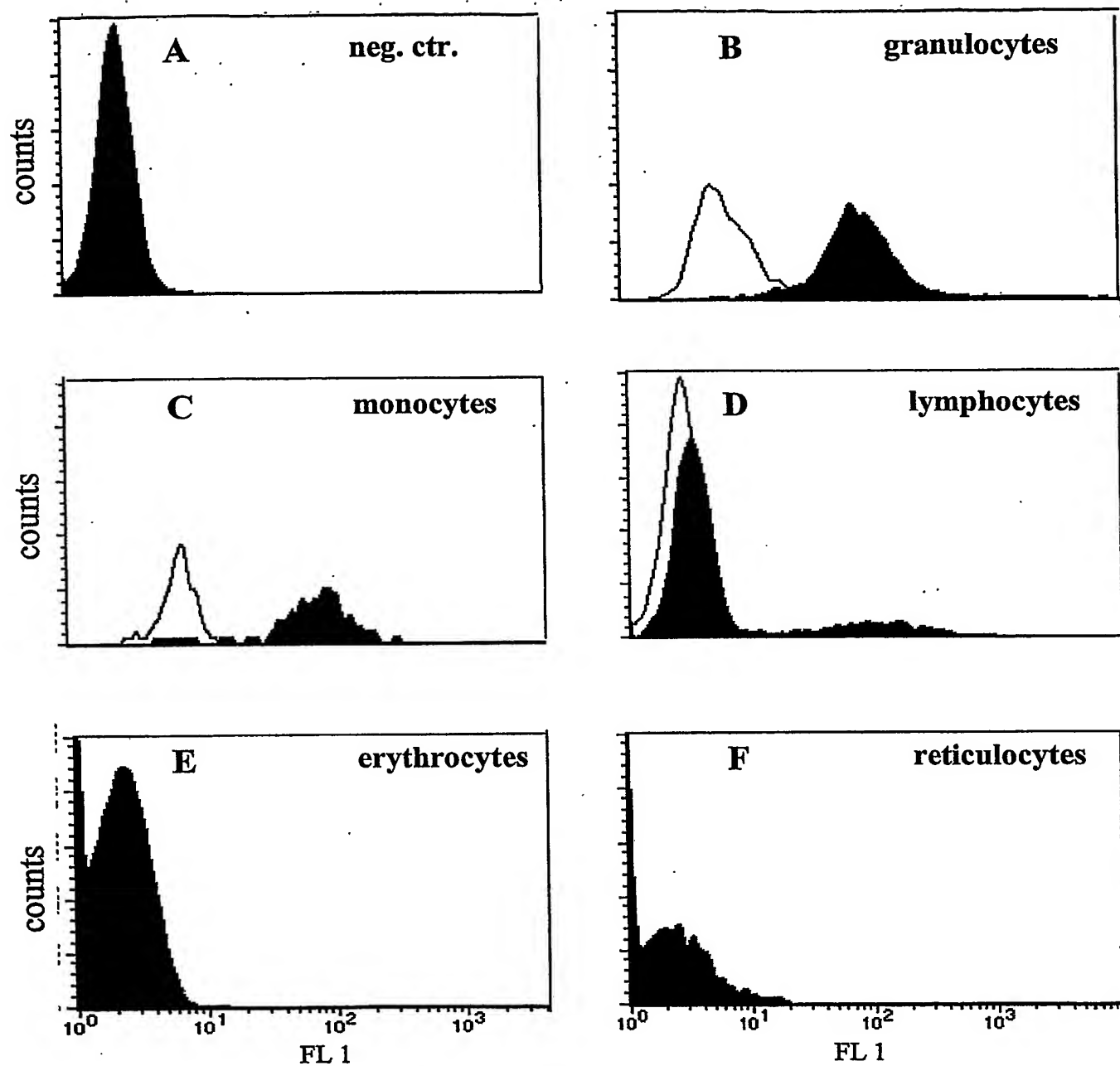


Fig. 8



**Fig. 9**

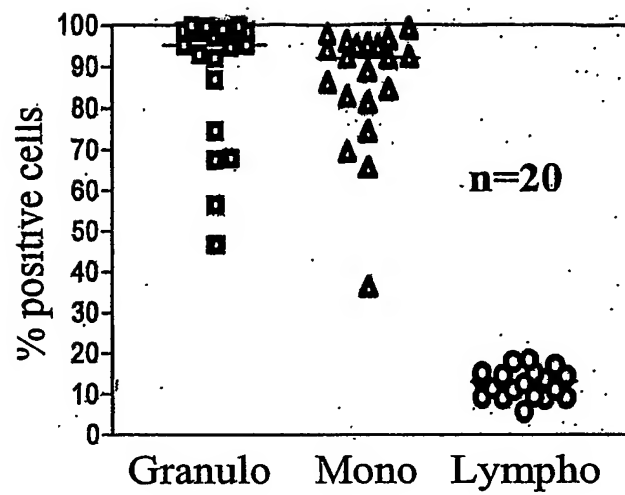
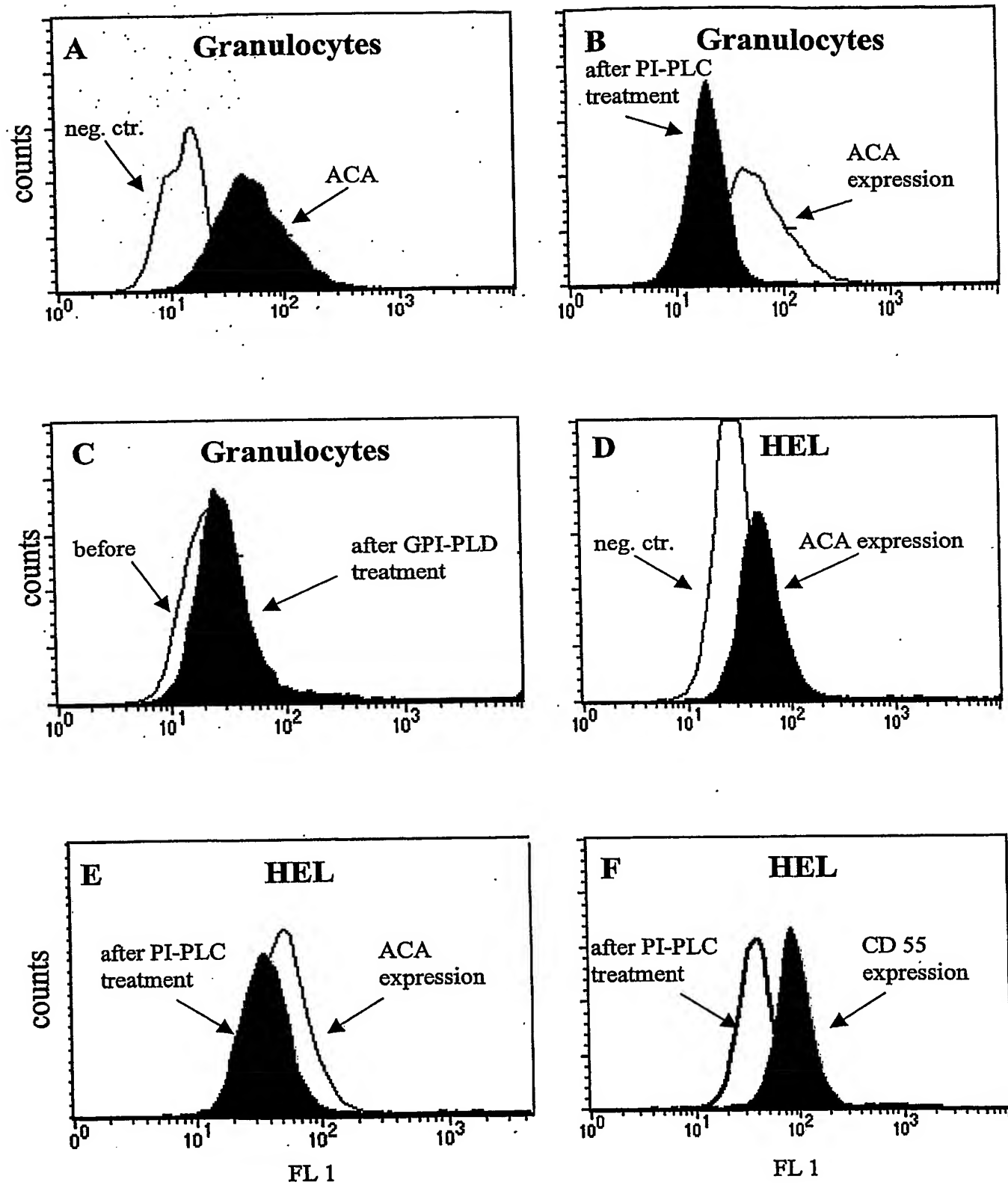
**Fig. 10**

Fig. 11



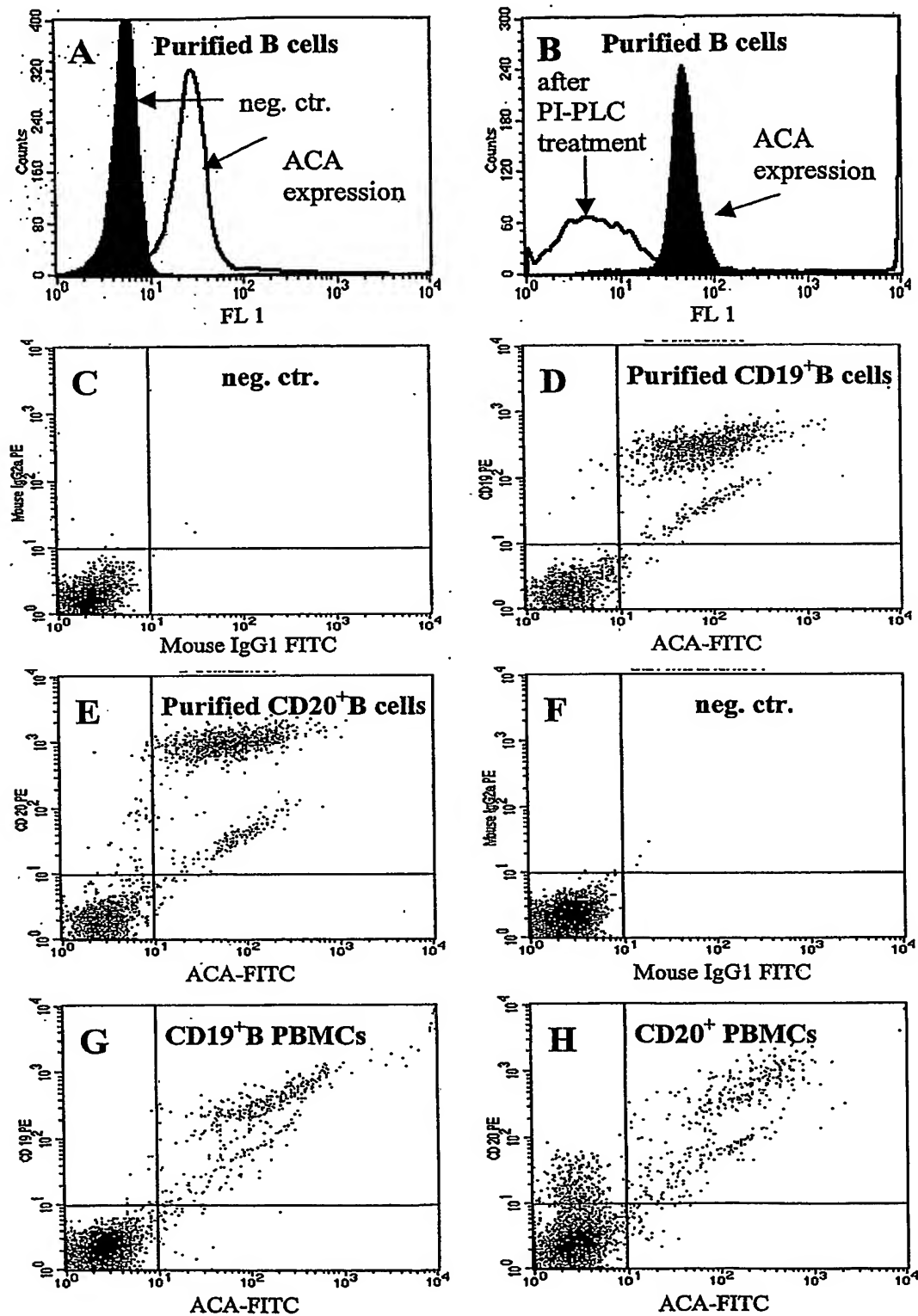
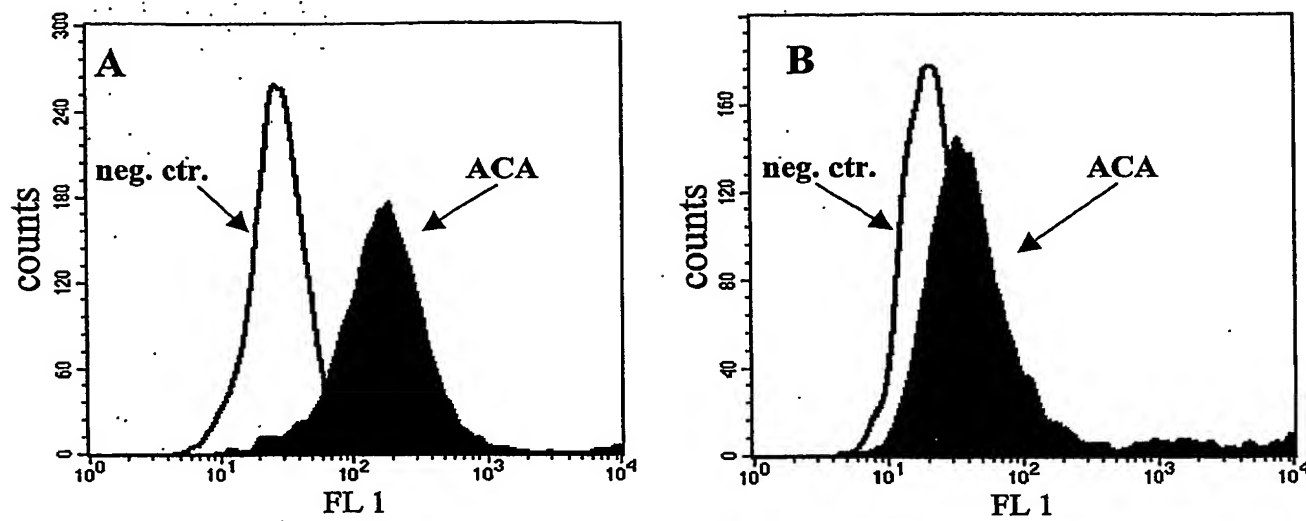


Fig. 12

**Fig. 13**

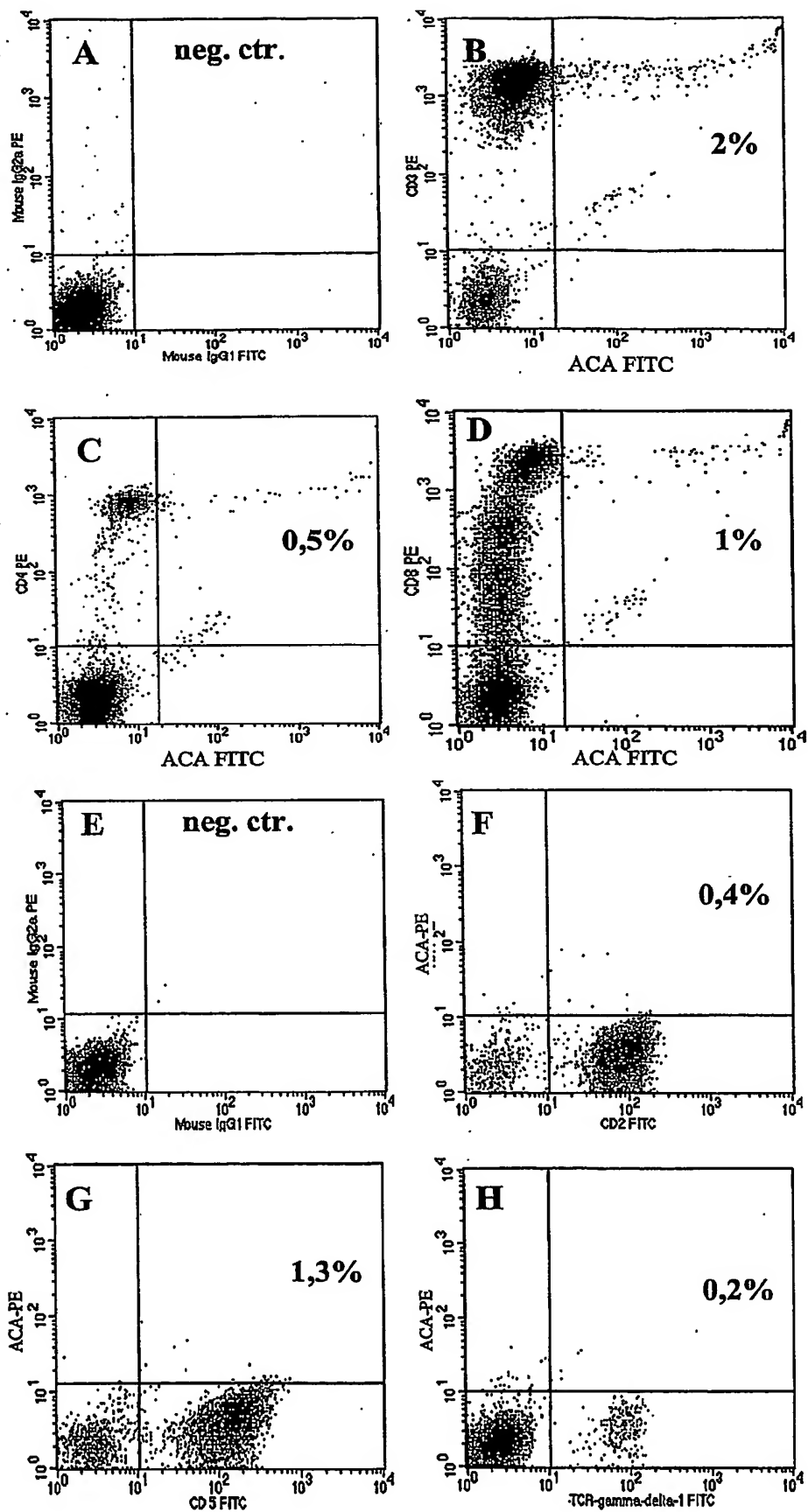


Fig. 14

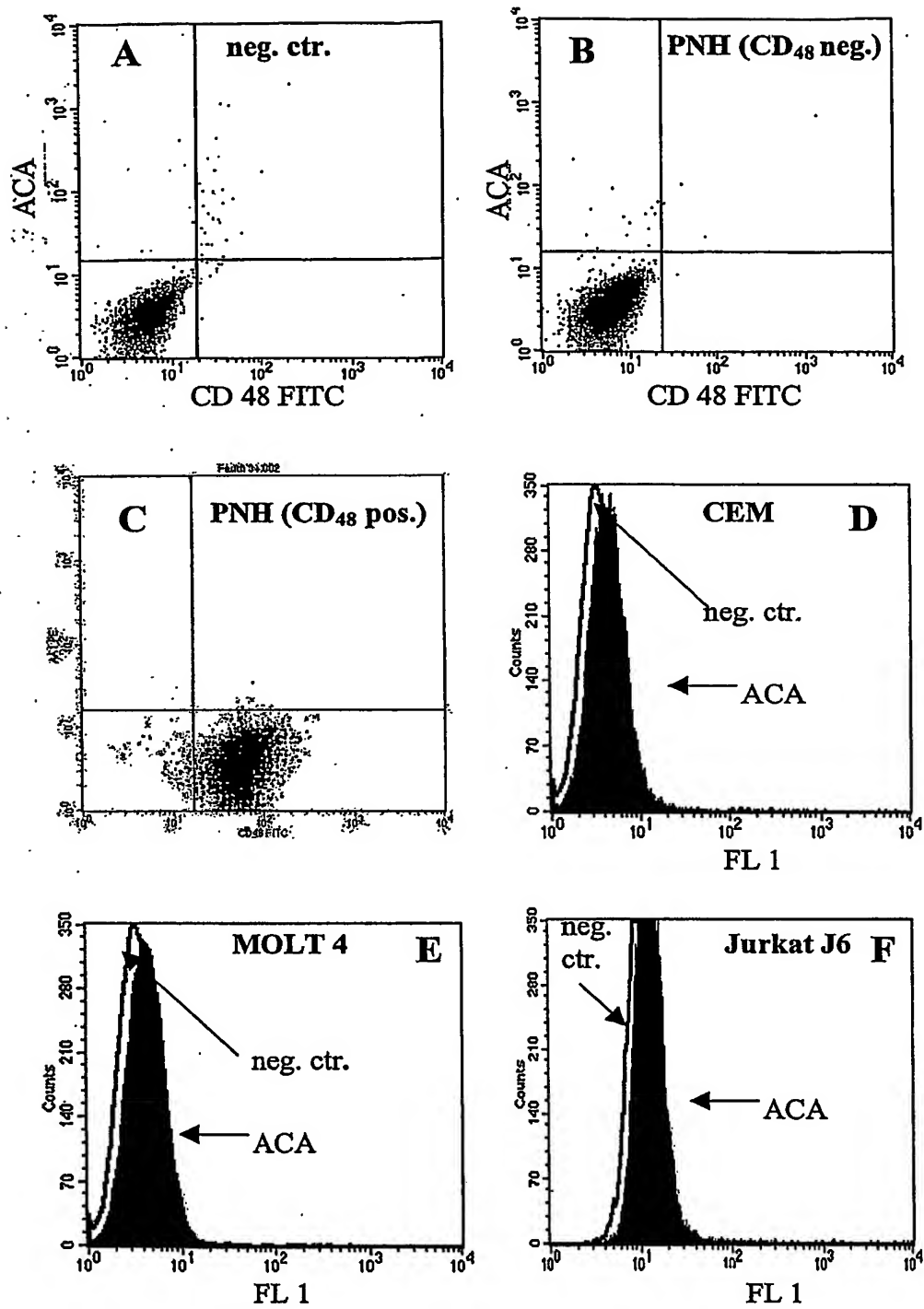


Fig. 15

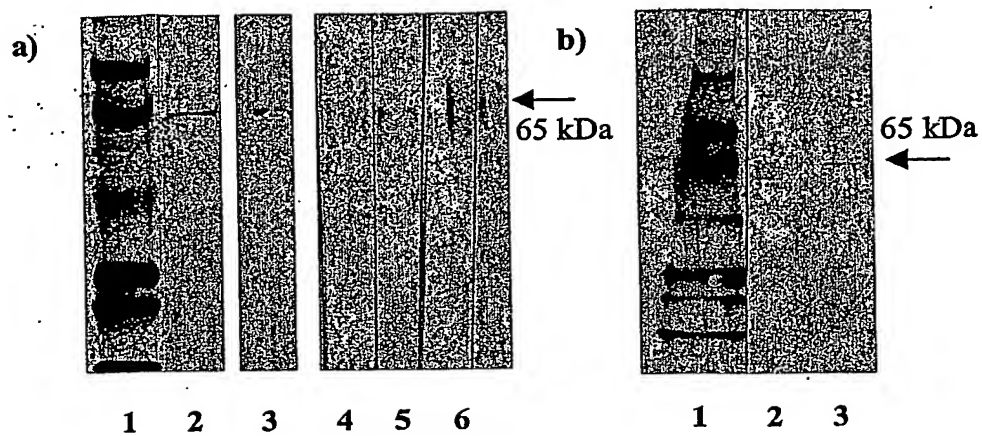
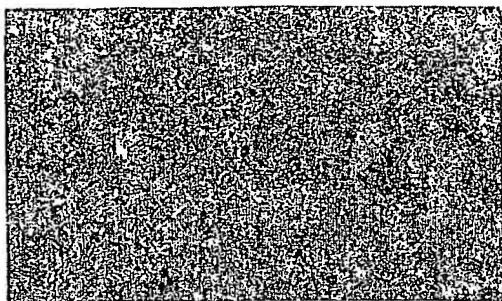


Fig. 16

Fig. 17

a)



b)

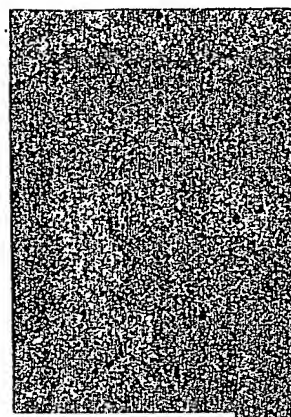


Fig. 17: Frozen sections of normal human skin stained with anti-ACA antibody.

Fig. 18

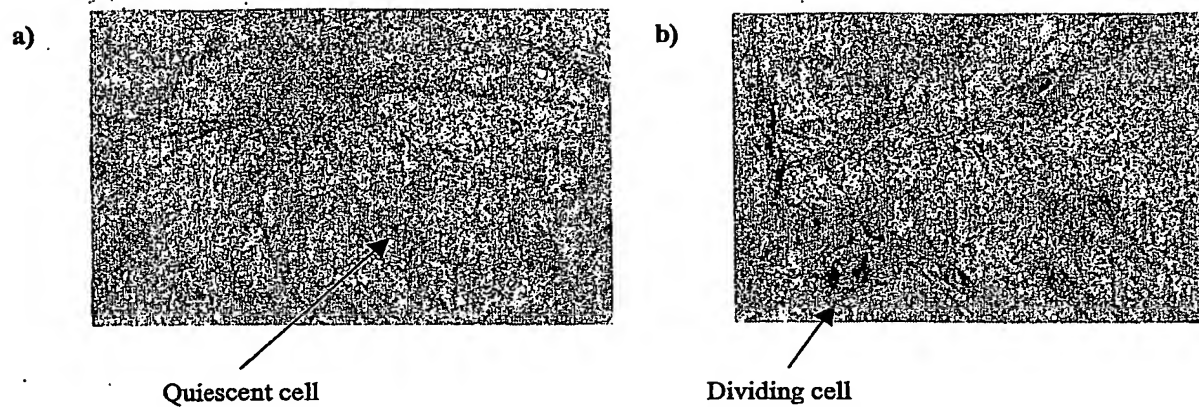


Fig. 18: Normal human cultured epidermal melanocytes stained with anti-ACA antibody.

Fig. 19 a/b

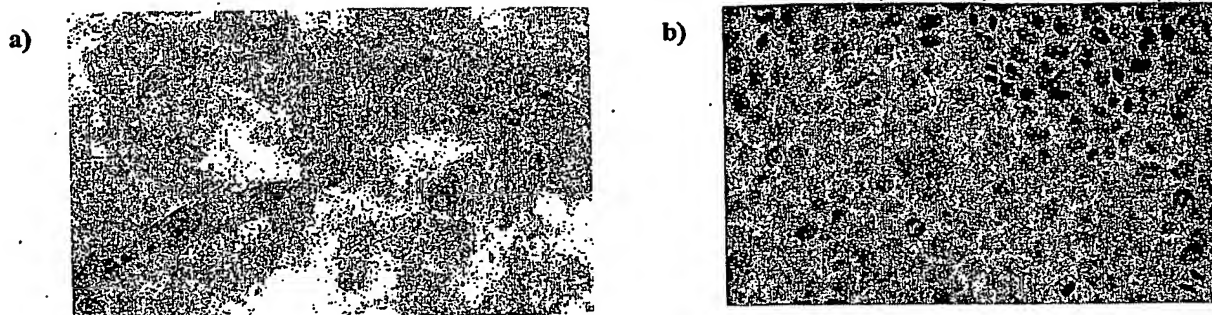


Fig. 19: Normal human cultured epidermal keratinocytes (a) and keratinocytes cell line HaCaT (b) stained with anti-ACA antibody.

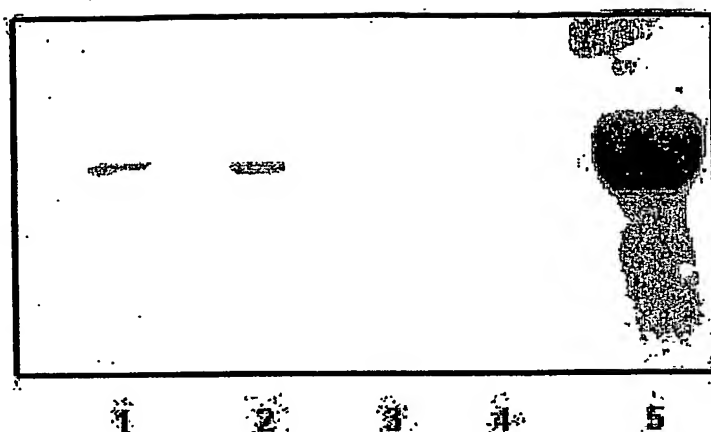


Fig. 19c: Immuno-blot analysis with anti-ACA antibody of cell-free extracts of melanocytes (lanes 1 and 2), keratinocytes (lanes 3 and 4), molecular weight marker (lane 5).

Fig. 20

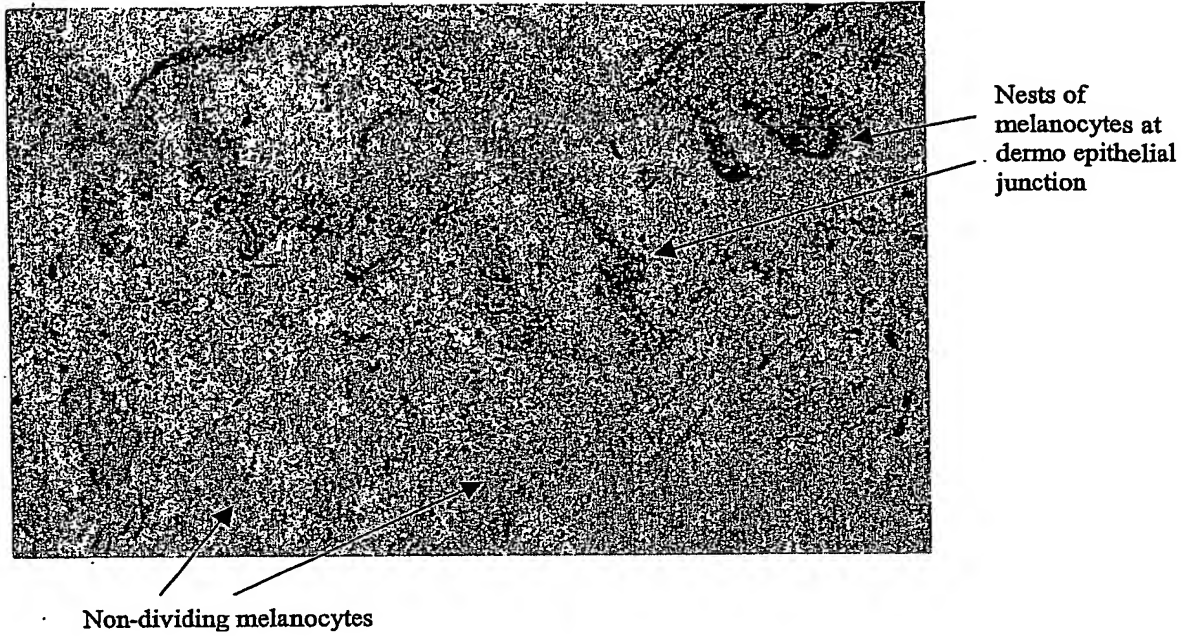
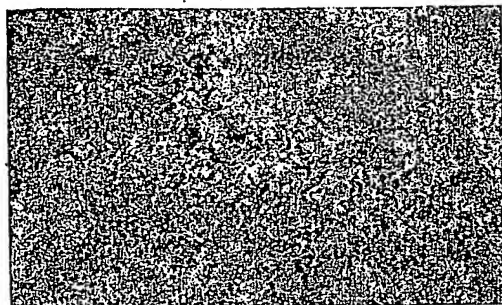


Fig. 20: Congenital Naevus

Fig. 21



b)

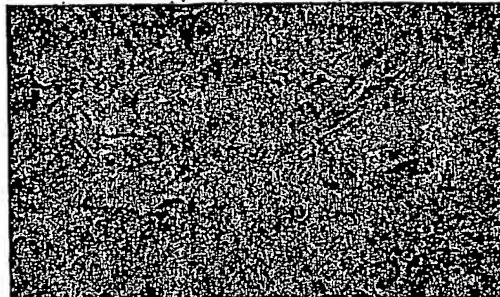


Fig. 21: Frozen sections of human melanoma stained with anti-ACA antibody

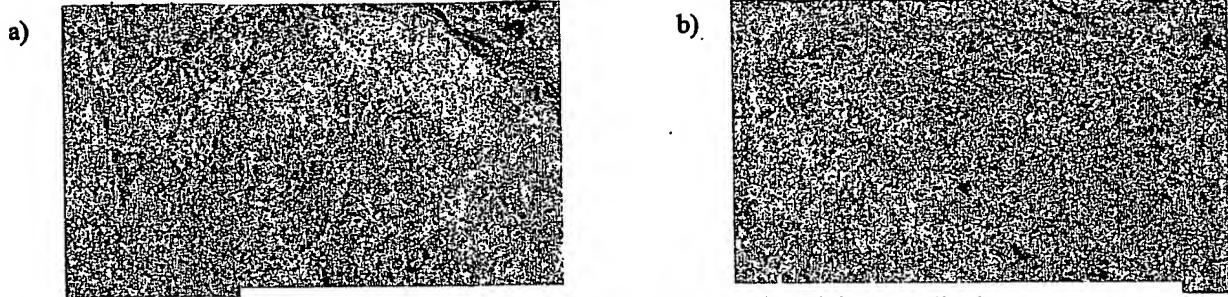
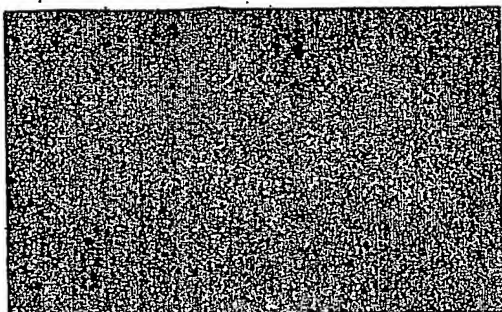
Fig. 22**Fig. 22: Melanoma skin metastasis stained with anti-ACA antibody.**

Fig. 23

a)



b)

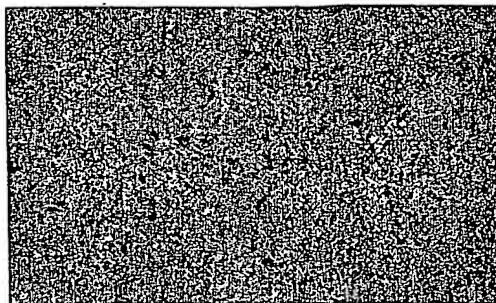


Fig. 23: Frozen sections of human a) basalioma, b) spinalioma stained with anti-ACA antibody.

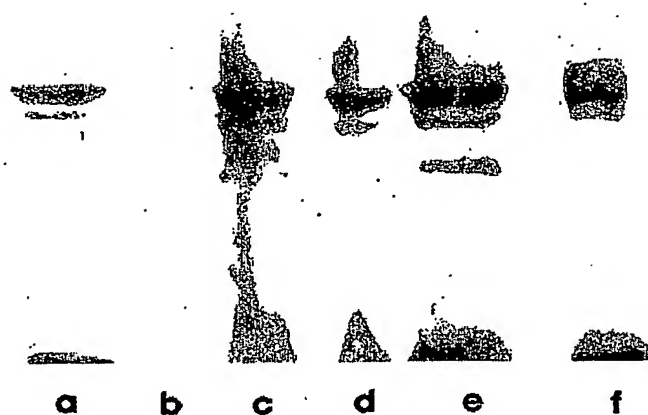
Fig. 24

Fig.24: Immunoblot analysis of normal skin and melanoma tumour tissues with anti-ACA antibody:
Homogenized normal skin (a), non-immune IgG (neg. ctr.) (b), homogenized melanoma tumour tissues
obtained from different patients (c-f).

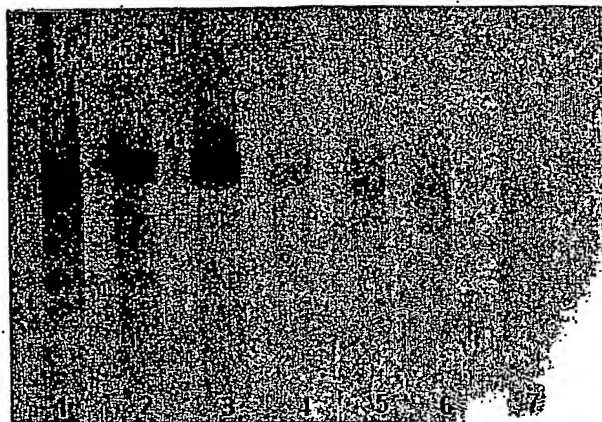
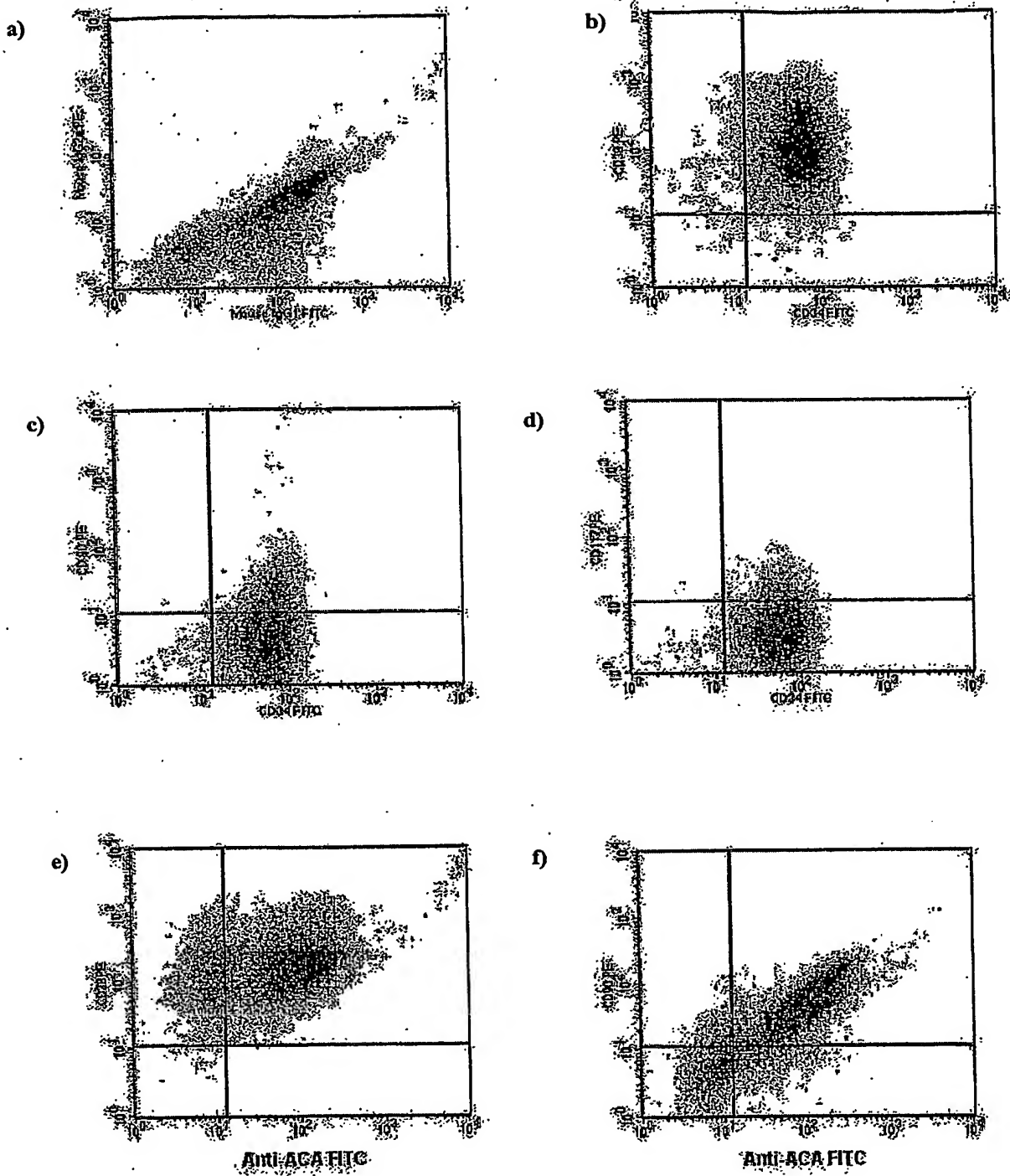
Fig. 25

Fig. 25: Immunoblot analysis of homogenized tumour tissues with anti-ACA antibody: Renal (1), Lung (2), Breast (3), Colon (4), Gastric cancer (5), Melanoma (6) and Myeloma (7).

Fig. 26



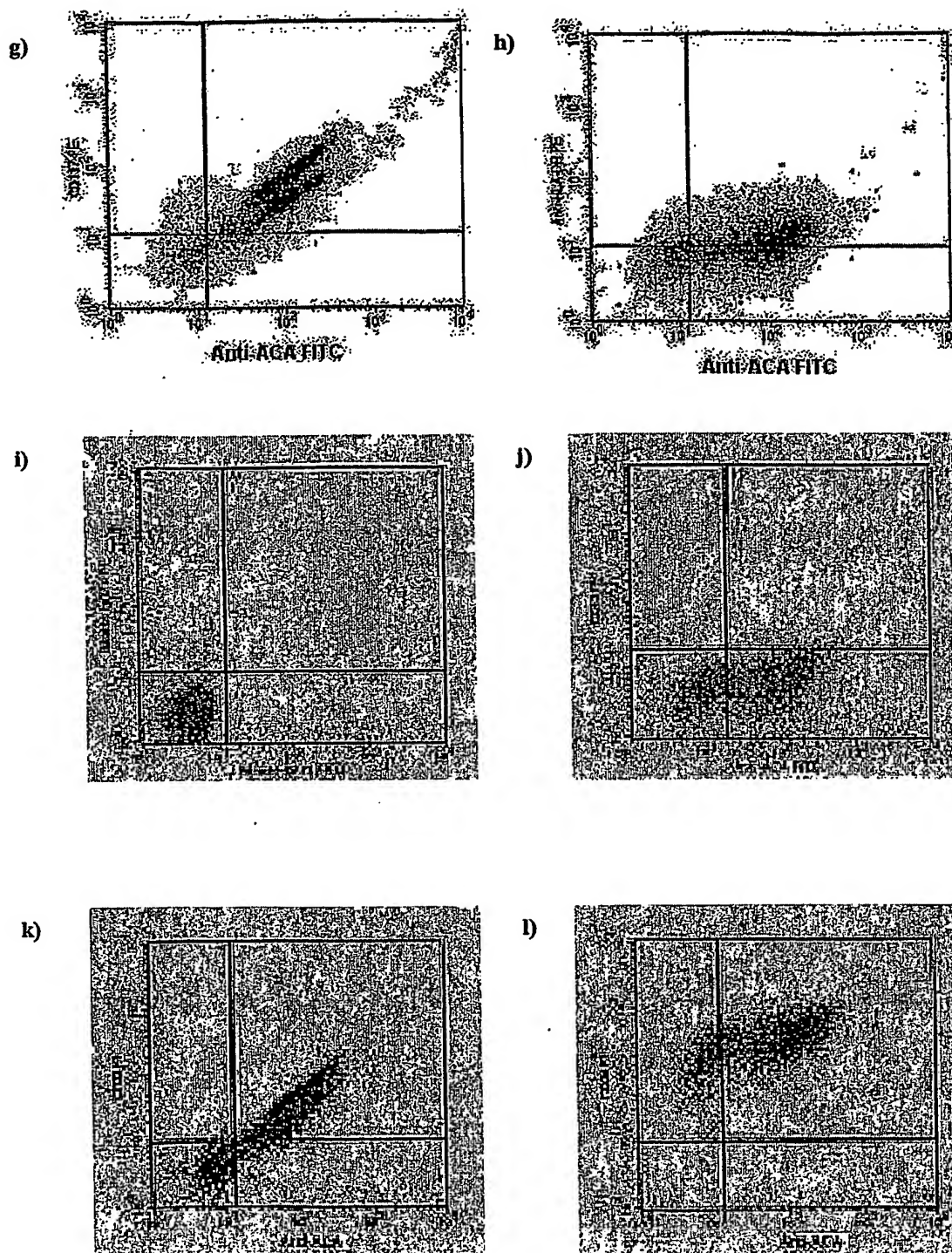


Fig.26: ACA is expressed on stem cell: non-immune IgG (a), CD34/CD38 (b), CD34/CD90 (c), CD34/CD117 (d), anti-ACA/CD38 (e), anti-ACA/CD90 (f), anti-ACA/CD117 (g), anti-ACA/HLA-DR (h), non-immune IgG (i), anti-ACA/CD13 (j), anti-ACA/CD33 (k), anti-ACA/CD34 (l).

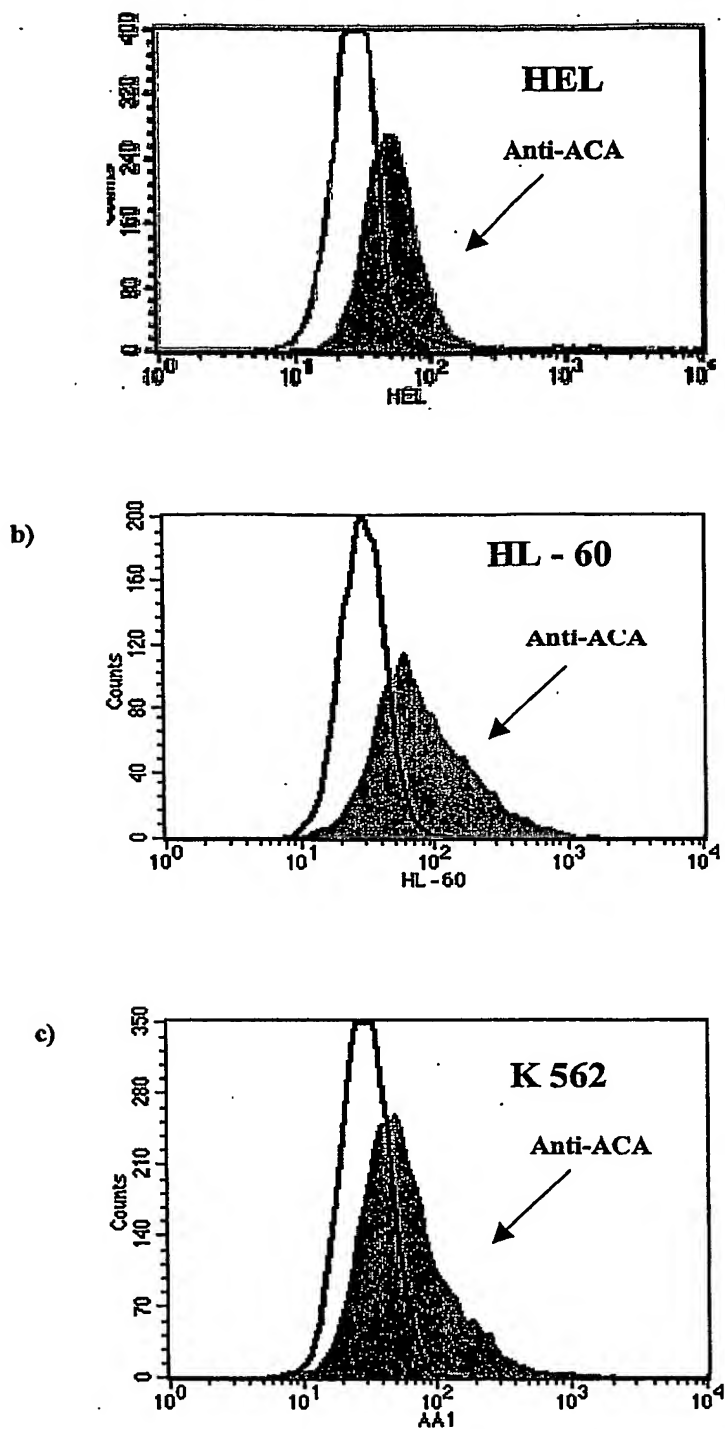
Fig. 27

Fig. 27 Flow cytometry analysis of ACA – expression on human leukemia cell lines: (a) erythroleukemia (HEL), (b) promyelocytic erythroleukemia (HL- 60), (c) chronic myelogenous leukemia (K562).

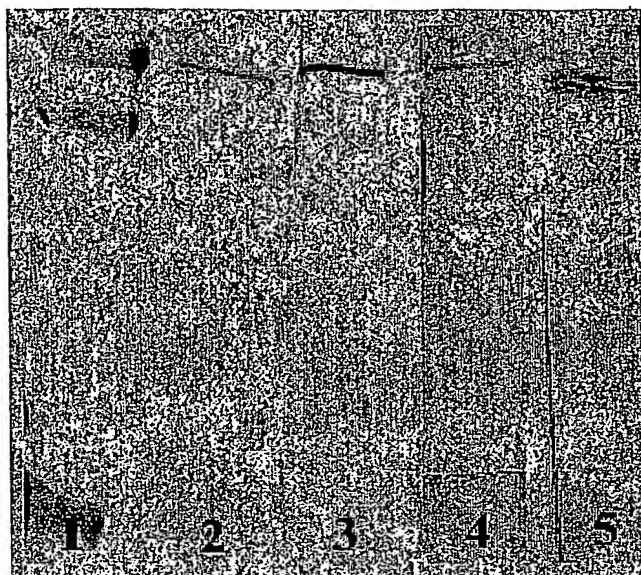
Fig. 28

Fig. 28: Immunoblot analysis with anti-ACA antibodies of cell-free extracts of human leukemia cell lines: molecular weight marker (1), human chronic myelogenous leukaemia (k-562) (2), human promyelocytic erythroleukemia (HL-60) (3), human erythroleukemia (HEL) (4), human histiocytic lymphoma (U-937) (5).

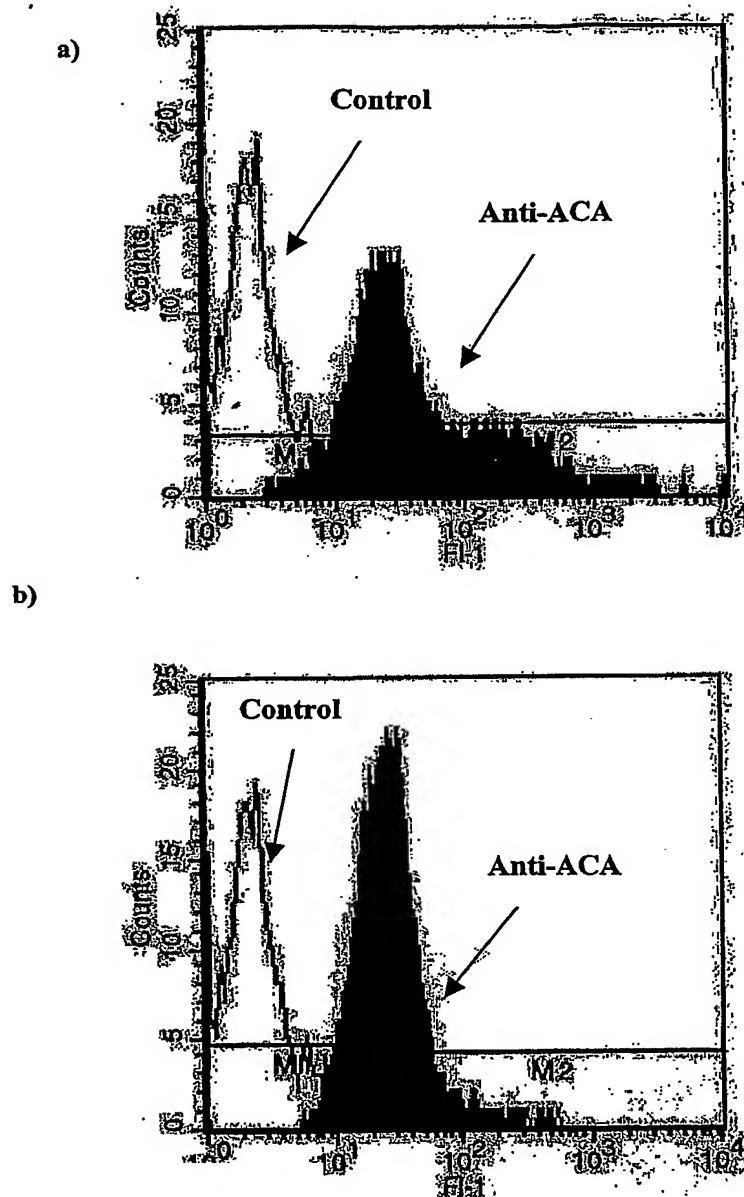
Fig. 29 a/b

Fig. 29a/b: Peripheral blood cells of PNH patients were incubated with mouse monoclonal antibodies to ACA plus anti-mouse-IgG FITC and analysed by FACS. After lysis of erythrocytes the granulocytes were gated by FSC/SSC.

Fig. 29 c

c)

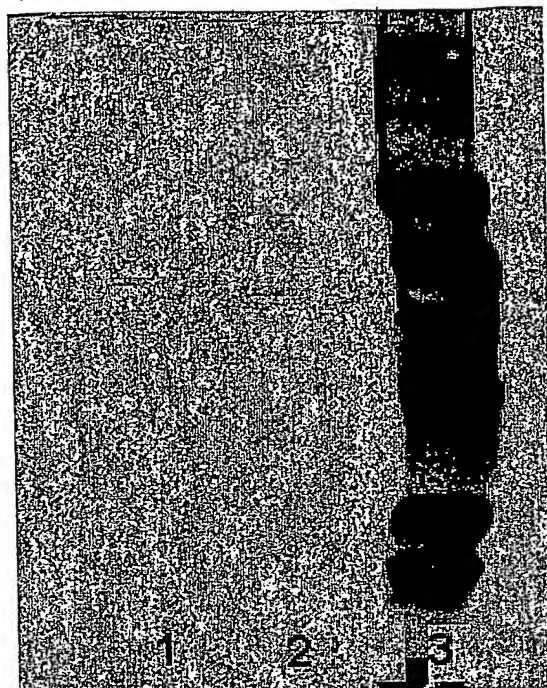


Fig. 29c: Immunoblot analysis with anti-ACA antibody of granulocytes membrane protein fraction obtained from healthy donor (lane 1), versus PNH patient (lane2).

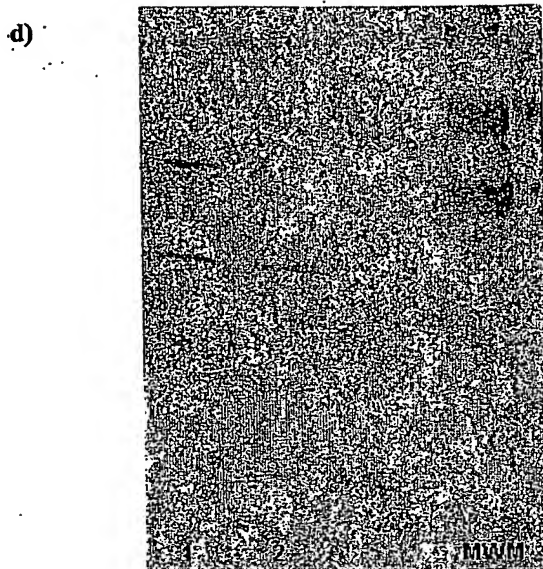
Fig. 29 d

Fig. 29d: Immunoblot analysis with anti-ACA antibody of PNH granulocytes membrane fraction before (lane 1), and after treatment with phospholipase C (lane 2), molecular weight marker (lane 3).

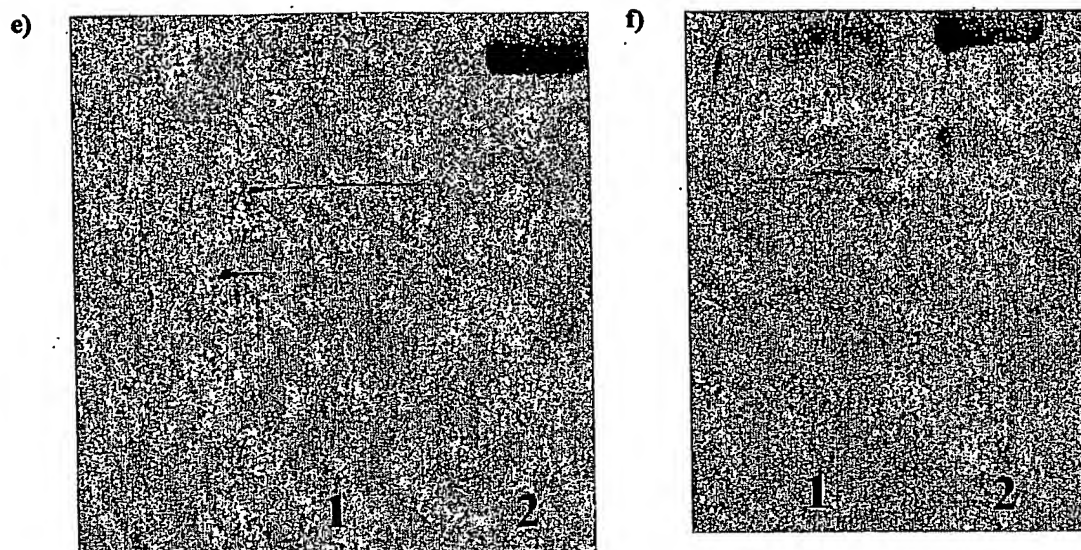
Fig. 29 e/f

Fig. 29 e,f: Immunoblot analysis of soluble form of ACA in supernatant of granulocytes derived from healthy donor (d) and PNH patient (e) after the action of phospholipase C.

34 / 39

Fig. 30**2 1**

Fig.30 Purification and isolation of ACA proteins. Electrophoresis of purified ACA proteins was performed on 4-15% SDS-PAGE. *Lane 1*, purified main form of ACA protein, molecular mass 65 kD; *lane 2*, purified ACA protein molecular mass 68 kD.

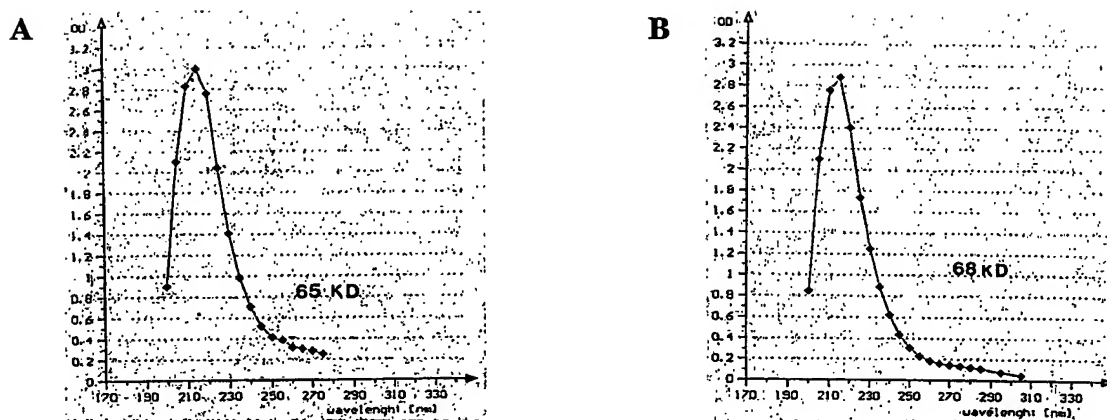
Fig. 31

Fig. 31 UV spectrum of isolated ACA proteins. UV spectrum of purified two forms of erythrocyte ACA protein was read in Beckman spectrophotometer. A, UV spectrum of purified 65 kD ACA protein; B, UV spectrum of 68 kD ACA protein.

36 / 39

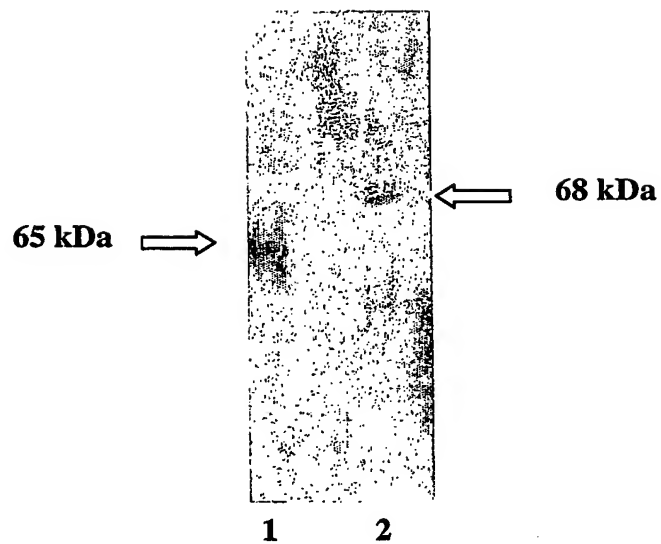
Fig. 32

Fig. 32 Western blotting with anti-ACA polyclonal antibody
Proteins were subjected to SDS-PAGE under reducing condition transferred to nitrocellulose, and revealed with mouse antibody to ACA. *Lane 1*, purified 65 kD erythrocyte ACA protein; *lane 2*, purified 68 kD erythrocyte ACA protein

37 / 39

Fig. 33

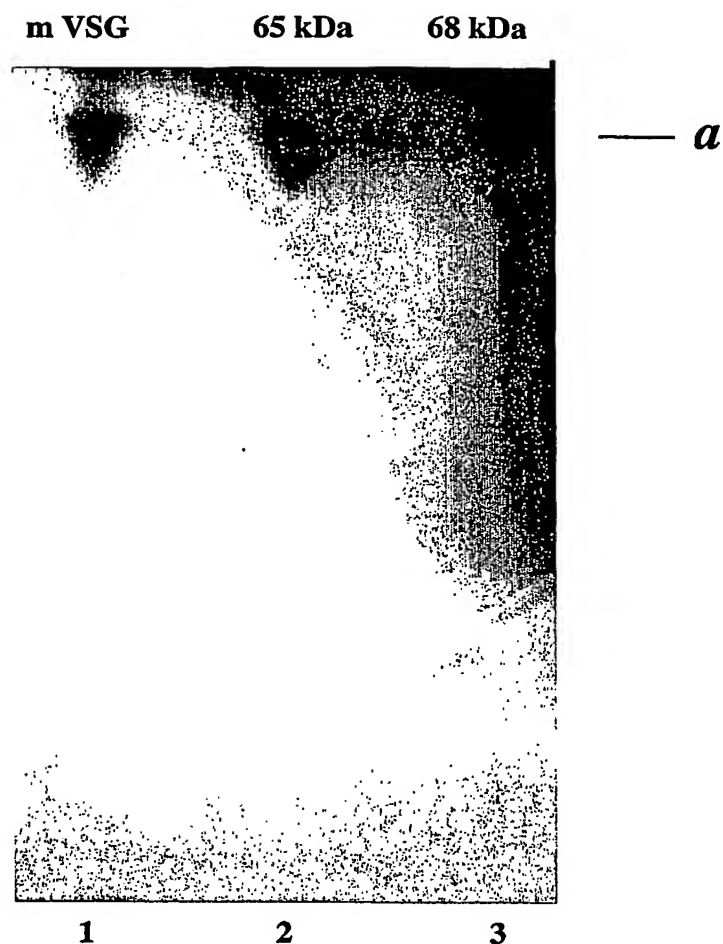


Fig. 33 Silica TLC analysis of fragments generated by hydrolysis of anchors. Samples of [125 I] TID-labeled, purified proteins were hydrolyzed with GPI-PLC. The lipid products of this reaction were further hydrolyzed with highly specific lipases. Radiolabeled fragments were extracted and analyzed by TLC. Myristic acid was used as standard. *Lane 1*, commercially available mVSG used as control was labeled, digested with GPI-PLC and further hydrolyzed as described for ACA; *lane 2*, 65 kD ACA protein digested with GPI-PLC and further hydrolyzed; *lane 3*, 68 kD molecular mass form of ACA digested and further hydrolyzed as already described.

38 / 39

Fig. 34

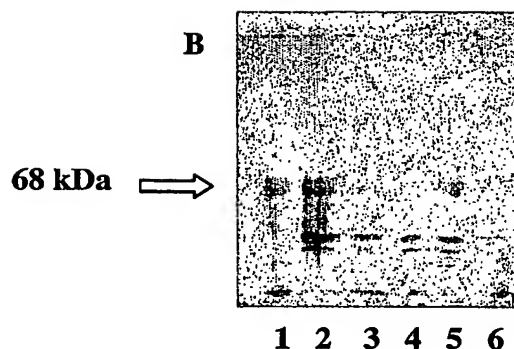


Fig. 34B SDS-PAGE analysis of 68 kD erythrocyte ACA after incubation with PNGase F according to Material and Methods. Lane 1 purified 68 kD ACA after incubation with PNGase F for 0 min; *lane 2*, purified 68 kD ACA protein incubated with PNGase F for 10 min; *lane 3*, 30 min; *lane 4*, 90 min; *lane 5*, for 150 min; *lane 6*, overnight.

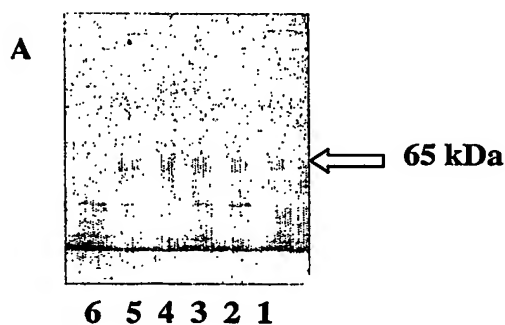


Fig. 34A SDS-PAGE analysis of 65 kD erythrocyte ACA after incubation with PNGase F according to Material and Methods. Lane 1 purified 65 kD ACA after incubation with PNGase F for 0 min, *lane 2*, purified 65 kD ACA protein incubated with PNGase F for 10 min, *lane 3*, 30 min, *lane 4*, 90mins, *lane 5*, for 150 min, *lane 6*, overnight.

39 / 39

Fig. 35

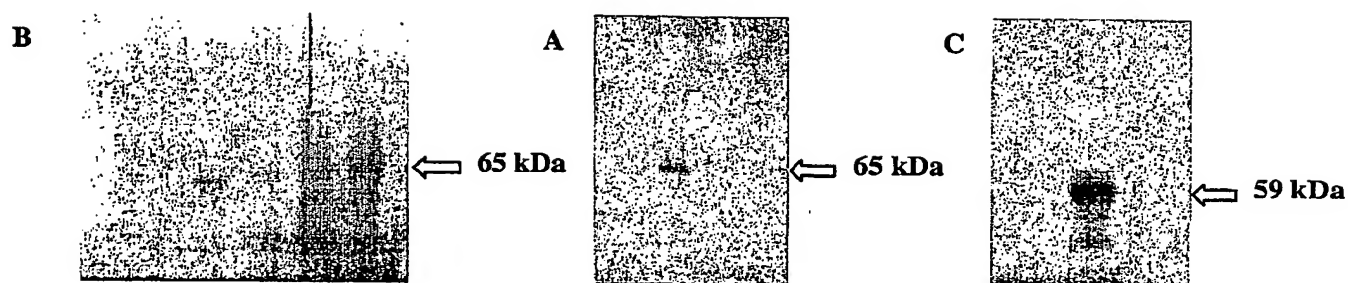


Fig. 35 Enzymatic and chemical deglycosylation of purified 65 kD erythrocyte ACA. Electrophoresis of purified 65 kD ACA protein and the products of its enzymatic and chemical deglycosilations was performed on 4-15% SDS-PAGE. *A, lane 1*, purified 65 kD ACA. *B, lane 1*, purified 65 kD ACA treated with sialidase, *lane 2*, O-glycosidase, *lane 3*, PNGase F, *lane 4*, subsequent treatment of 65 kD erythrocyte ACA with sialidase O-glycosidase and PNGaseF. *C, lane 1* purified 65 kD ACA protein after a chemical deglycosilation using TMSF.